

**A. Cover Sheet (Attach to front of proposal)**

W0201-0022

1. Specify: ☒ agricultural project or ☐ individual application or  
☐ urban project ☐ joint application

2. Proposal title—concise but descriptive:

**On Site Demonstration of Field Bioremediation Strategies to Reduce Selenium Levels in Drainage Sediment**

3. Principal applicant—organization or affiliation:

**Dr. Gary S. Bañuelos and Dr. Shankar Sharmasarkar (Water Management Research Laboratory, USDA-ARS, Fresno)**

4. Contact—name, title:

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9. Funds requested—dollar amount:

\$255,538

10. Applicant cost share funds pledged—dollar amount:

\$100,000 plus inhouse money from USDA

11. Duration—(month/year to month/year):

July 2001 to June 2004

12. State Assembly and State districts and Congressional district(s) where the project is to be conducted:

Congressional district 20; Senate district 16; State Assembly district 31

13. Location and geographic boundary of the project:

San Joaquin Valley Agricultural Research Center, Parlier, CA - Field Site #32

14. Name and signature of official representing applicant. By signing below, the applicant declares the following:

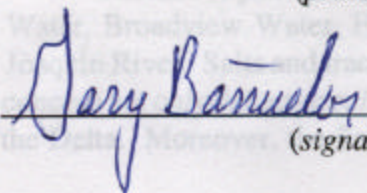
- the truthfulness of all representations in the proposal;
- the individual signing the form is authorized to submit the application on behalf of the applicant;
- the applicant will comply with contract terms and conditions identified in Section 11 of this PSP.

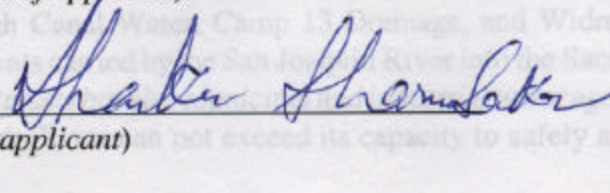
**GARY S. BAÑUELOS and SHANKAR SHARMASARKAR**

**February 14, 2001**

(printed name of applicant)

(date)

  
(signature of applicant)

  
(signature of applicant)

# On Site Demonstration of Field Bioremediation Strategies to Reduce Selenium Levels in Drainage Sediment

Gary S. Bañuelos and Shankar Sharmasarkar

## B. Scope of Work

### 1. Abstract:

Scientific research is urgently needed to provide accurate knowledge of potential risks associated with contaminated soils and help regulatory agencies to make the best possible decisions concerning the need for remediation measures. Excessive rainfall, flooding, irrigation, drainage, and wind have contributed to the excessive accumulation of selenium-rich sediment in parts of the canal system within the San Luis Drain. These accumulated sediments are a tremendous source of salts, selenium (Se), and boron (B). Due to high costs associated with removing and transporting the 2 million plus cubic feet of sediment residing in the canal, the decision was made to leave the sediments in place. Scientists at the USDA-ARS-Water Management Research Laboratory propose setting up a demonstration field study to show that bioremediation involving plants, microbes, and water management, can naturally encourage the transformation of Se species within the sediment into forms that plants can take up and into forms that volatilize as a harmless gas. Two 0.5 ha field plots have been constructed at the new USDA research facility in Parlier, CA. In preparation for the study, four hundred cubic yards of drainage sediment was transported from San Luis Drain and spread to a depth of 25 cm on the excavated plots. A surface drip irrigation was installed and placed on the forty 33 m long and 100 cm beds. Perennial crops based upon salt and B tolerance (determined already in greenhouse study) and their economical viability as forage crops, will be planted on the sediment-applied soil. Soil samples will be incrementally collected at preplant and harvest to a depth of 1.5 m and chemically and biologically evaluated for transformed species of Se and their respective movement and volatilization within the soil profile over time and over depth. Periodic plant sampling will provide yield information, as well as data on the plant accumulation of Se and trace elements over time. Relationships between plant, microbial activity, water management and their effects on transforming Se into volatile forms of Se will be evaluated for each respective plant species. Depending on plant Se concentrations, harvested Se-enriched plant material will be blended with non-Se containing alfalfa (to dilute plant Se concentrations) and fed as a source of animal forage to rabbits raised on site; Se is an essential micronutrient for biological systems. By using this bioremediation strategy to reduce the toxic concentrations of Se in the sediment, it is our objective to demonstrate an alternative method for naturally detoxifying the Se-laden sediment residing in parts of the San Luis Drain.

### 2. Rationale Statement of Issues:

The western San Joaquin Valley is arid and typically receives too little rainfall to make commercial agriculture viable without supplemental irrigation. Infiltrating irrigation water dissolves salts and trace elements and leaches them into the shallow groundwater. Where drainage is provided, it typically contains high concentrations of dissolved solids, selenium (Se) and boron (B). In the western San Joaquin Valley, most of the inorganic salts contained in drainage water originate from groundwater displaced into drainage tiles by infiltrating irrigation water.

Historically the six agricultural water districts on the westside of the SJV (Charleston Drainage, Pacheco Water, Broadview Water, Firebaugh Canal Water, Camp 13 Drainage, and Widren Water) drain to the San Joaquin River. Salts and trace elements carried by the San Joaquin River into the Sacramento/ San Joaquin Delta concern not only delta agricultural interests but also municipal and industrial water agencies that divert water from the Delta. Moreover, the San Joaquin River can not exceed its capacity to safely accommodate contaminants

without exceeding water-quality concentrations established by the State Water Resources Control Board.

Interim use of a 28-mile section of the San Luis Drain was approved as a first step in 1985 toward alleviating chronic water-management problems for wildlife area in the greater Grasslands Basin; this was known as the Grasslands Bypass Project. Use of the San Luis Drain was responsible for much of the accumulated sediment along the 28-miles drain alignment. Excessive rainfall, flooding, irrigation, drainage, and even wind contributed to the movement of soil particles high in Se from near-lying fields to parts of the drainage canal. Continued use of the San Luis Drain was responsible for the accumulated sediment upstream and downstream along the drain alignment. The prohibitive cost of removing and transporting the approximate 2 million plus cubic feet of sediment (estimated at between \$1.1 million and \$3.4 million led to the decision to leave the sediments in place. These accumulated sediments are a tremendous source of salts, Se, and B. Electrical conductivity values are as high as 45-50 dS/m, while concentrations of total sediment Se are 75 mg/kg and of extractable B are as high as 45 mg/L. If the inorganic salts were mobilized and reintroduced into the water solution, there is concern that the concentrations would exceed hazardous materials concentration. Presently, there are no practical and inexpensive strategies available for disposing or managing the Se-laden sediment.

After more than 15 years of extensive research on reducing Se levels in soil and water, the USDA-Water Management Research Lab developed a new strategy for naturally bioremediating Se. Utilizing selected plant species, they were able to successfully lowers Se levels in the soil environment by plant accumulation and volatilization (Bañuelos and Schrale, 1989; Bañuelos and Meek, 1990; Bañuelos et al. 1993; Bañuelos et al. 1993b, 1993c, Bañuelos et al. 1996; Bañuelos et al. 1997; Bañuelos et al. 1998., and Bañuelos 2000).

Their field research with bioremediation on 3-Way Farms, Los Banos, CA; Red Rock Ranch, Five Points, CA; and most recently with Broadview Water District, Firebaugh, CA shows that growing special crops to reduce Se levels in soils requires a wide range of knowledge about the chemistry and transformation of Se in soil, Se uptake by plants under high saline and B conditions, and sustainable agronomic practices for long-term crop production. Based upon recent greenhouse studies conducted with drainage sediment collected from the San Luis Drain in Broadview Water District (primarily deposited from the flooding of Silver Creek and near-lying Se-rich, agricultural soils), the WMRL successfully identified plant species that tolerated growing in the extreme conditions of the sediment. Not only were the tested plants extracting and accumulating Se up to 13 mg/Kg DM after three months, but biological volatilization of Se was also measured up to 150 µg/day. Both accumulation and volatilization of Se by the plants will contribute to a lowering of Se levels in the sediment. Because plants used for bioremediation of Se are a harvestable commodity, disposal options must be considered. Bañuelos and Mayland (2000) recently demonstrated that harvested materials from plants used for Se remediation can be utilized as a source of supplemental Se for sheep and dairy cows in central California (Se is an essential micronutrient for livestock production!). Our **hypothesis** is that *bioremediation, an inexpensive and proposed new technology, will provide water districts in the westside of central California with a natural technique for managing toxic levels of Se in sediment residing in different sections of the San Luis Drain.*

Based upon our experience acquired in the field and cooperation with growers regarding related bioremediation projects, the WMRL has identified essential factors to consider in order for Se remediation to occur in drainage sediment. These include:

- (1) soil salinity and high concentrations of toxic elements;
- (2) presence of competitive ions affecting Se uptake;
- (3) water management strategies that produce less effluent; and
- (4) acceptance of bioremediation as a remediation technology for Se-laden sediment by the public, regulatory agencies, and by Water Districts in the westside of SJV having sections of the San Luis Drain.

### 3. Nature, Scope, Objectives:

The project will benefit Charleston Drainage, Pacheco Water, Broadview Water, Firebaugh Canal Water, Camp 13 Drainage, and Widren Water District, Regions 15, 16, 17.

We are proposing the use of bioremediation for lowering the Se levels in sediment residing in drainage canals in the west side of central California. Perennial plant species will be planted in Se-laden sediment transported from accessible portions of the San Luis Drain, redeposited at the USDA research facilities in Parlier, CA and then evaluated for reducing Se levels in the sediment.

The **Objectives** of this multi-year demonstration field study are to:

- (1) evaluate plant uptake of Se and B grown in excavated drainage sediment;
- (2) examine the use of surface drip irrigation for promoting the remediation of Se in the sediment;
- (3) quantify distribution, movement and transformation of Se and B (in soils and plants) under the integrated microirrigation and bioremediation approach using chemical and microbiological assessments;
- (4) assess biological volatilization of Se; and
- (5) determine effectiveness of both plant uptake and microbial activity for lowering total and soluble sediment Se concentrations to an environmentally safe level.

### 4. Materials, Methods and Monitoring:

#### 4.1. Field Study Design

We have already excavated sediment, from the San Luis Drainage Canal, allowed it to semi-dry on the sides of the canal, and then transported it to our new USDA research site in Parlier, CA, where the study will be conducted undisturbed for many years. Two 33 x 33 m field research plots with twenty 33 m long and 100 cm width beds, respectively, have already been constructed. Four hundred cubic yards of drainage sediment (collected at same site described above) have been spread to a depth of 25 cm in a previously excavated 33 x 33 m treatment plot, while the "control plot" does not contain sediment. A surface drip irrigation system has been installed consisting of in-line turbulent flow emitters with a 4 L/h emitter discharge rate and emitter spacing of 0.45 m. The CIMIS weather station located at Kearney, Parlier, CA will be used to help determine irrigation frequency and used to approximate crop coefficients for alternative crops evaluated. Crops have been selected based upon their salt and B tolerance already observed in the greenhouse study, and for their potential economical viability as forage crops.

Treatments will consist of planting at least four plant species on separate beds and having a bare plot (no plants), with four replications per treatment. Growing seasons will be determined depending on the chosen species. Normal agronomic management will be practiced, including interval clipping of plants throughout the growing season. New plant species may be selected after observing growth parameters of previously planted species. Soil samples will be taken incrementally to a depth of 1.5 m at preplant and after harvest of each respective plant species. A minimum of eight composite soil samples will be collected from each species including bare plot, and four composite plant samples will be collected from four one m<sup>2</sup> sites per species.

## 4.2. Chemical and Biological Analyses

Distributions and movement of Se and B species in soils and absorption by plants, under the integrated management approach, will be estimated by **chemical** and **microbiological** assessments. Samples (plants, soils and leachates) will be collected periodically through the growing seasons and analyzed for various bioavailable Se and B species. Soil and soil-solution samples will be collected periodically every 3 weeks from 0-150 cm depth (rooting depth) of the irrigated plots and analyzed for Se and B. Plant samples will also be analyzed periodically for ion accumulation. Soil samples will be collected in 30 cm increments up to a depth of 150 cm (root zone) within each treatment plot using a 1-cm diam., 200-cm long auger.

After each sampling, soils will be air-dried, finely ground through a 2-mm sieve and stored at 4°C in polyethylene bags. All other samples will also be stored in polyethylene containers at 4°C until analysis. Selenium **speciation and distribution** over time at different soil depths will be utilized to determine **kinetics of leaching and plant-uptake**. We will also monitor **pH and redox potential (Eh)** in order to compare the **transformation** of Se with leaching. In each plot, neutron probe tubes and tensiometers will be installed to determine water content and matric potential. Data obtained from these two methods will be also used to verify the amounts of **crop water use** calculated from the weather data. After multiple clippings, plant materials will be analyzed for total **yield**, and **Se and B** accumulation. Undisturbed soil cores will be collected to measure bulk density and moisture content. *In situ* infiltration experiment will also be conducted to determine **infiltration rate, sorptivity and hydraulic conductivity**. Other **analytical parameters** that will be measured includes: **total and water extractable** concentrations of Se and B at incremental depths in the soil profile; and **volatile Se**.

Daily maximum and minimum air temperature, precipitation and evapotranspiration rate will also be recorded in order to determine the duration of water application, irrigation interval, and subsequent irrigation scheduling. Based on field capacity, wilting point, bulk density, rooting depth and maximum application efficiency the **net water requirement** will be calculated which will be utilized along with the peak evapotranspiration to determine irrigation interval. Using the pan method we shall also determine the **quantity of water applied** for various **depletion factors**. Finally, **agronomic water use efficiency (AWUE)** will be determined based on the total quantity of water received by the crops during the growing season and yield data.

Since Se mobility, transformation and volatilization will be affected by the degree of microbial activity (Frankenberger and Karlson, 1988; Zayed et al., 2000), we will determine the system **microbial biomass** and its influence on speciation dynamics. Total soil microbial biomass will be estimated using the chloroform fumigation-extraction and incubation method (Jenkinson and Powlson, 1976; Jenkinson, 1987; Horwath and Paul, 1994). This technique is based on lysis of microbial cells in soil by exposure to chloroform vapor and chemical extraction of liberated microbial nitrogen. Fungal biomass in soil will be determined using a combination of direct microscopic observation and the ergosterol method. Bacterial biomass will also be quantified by direct microscopic observation using fluorescein isothiocyanate (FITC) as stain. Selenium **volatilization** studies will be carried out in plexi-glass growth chambers comprising selected plant species grown in sediment/soil mixture treatments. Chambers will be constructed based upon preliminary design described by Lin et al. (1999). Volatilized organic Se in air will be sorbed periodically onto activated charcoal and quantified. Both plant and soil materials will be analyzed for inorganic, organic and total Se fractions in order to identify the percent of biological volatilization during the bioremediation process.

## 5. Facilities and Resources:

This collaborative greenhouse and field research studies between the Water Management Research Laboratory, west side Water Districts, growers of Se-laden soils in the west side, and CSU Fresno will utilize the strengths of each institution to jointly address the environmental issue of Se remediation in central California.

In our new USDA research facility at Parlier, CA (see enclosures), we have over 1500 sq ft. available as chemical laboratories. Electronic, soil physics, computer, and workshops are available for general use. Equipment relevant to this proposed study include all typical farm machinery (including Giddings Rig for soil sampling) necessary for any cultivation practice, large outdoor plant and soil drying ovens, wood-chippers for plant grinding large plant samples, soil grinder, four fume hoods and facilities for acid and ash digestion (for digestion blocks, muffle furnace, microwave digester), Perkin Elmer inductively coupled plasma spectrometer for all inorganic elements, Thermo Jarrell Ash atomic absorption spectrometer with an automatic vapor accessory for Se analyses, Dionex ion chromatograph for anions, titrimeter for chlorides, Alpkem autoanalyzer for nitrates and phosphates, elemental analyzer for total N, conductivity/salinity meters. Other relevant facilities include 2500sq ft of greenhouse space, two environmental growth chambers, sterilized hood, and autoclaves.

## 6. Schedule:

### Timeline for Three Year Project

Activity	2001-2002 JASONDJFMAMJ	2002-2003 JASONDJFMAMJ	2003-2004 JASONDJFMAMJ	2002-2003 JASONDJFMAMJ
<b>Volatile Se Chamber</b>				
Construction/testing	xx			
<b>Field</b>				
Prepare beds	xx			
Check irrigation	xx			
Sample soils	x	x	x	x
Plant	x	x	x	x
Determine water rates	x	x	x	x
Sample plants	x-----x	x-----x	x-----x	x-----x
Setup Se chambers	x-----x	x-----x	x-----x	x-----x
Analyze samples	x-----x	x-----x	x-----x	x-----x
<b>Demonstration</b>				
Demonstrate field operations		x	x	x
<b>Data</b>				
Process	x-----x	x-----x	x-----x	x-----x
<b>Reports</b>				
Interim	x	x	x	x
Annuals	x	x	x	x
Final				x-----x

### C. Outreach, Community Involvement, and Information Transfer

1. Gary Banuelos, the head PI, actively participates in outreach efforts to involve participation from students in disadvantaged communities. In this regard, he has been selected to represent the USDA as a "model Hispanic scientist" since 1990. He actively recruits Hispanic, Native Americans, and underrepresented students for summer employment at the USDA Research Unit in Fresno, CA. In addition, he attends minority student conferences throughout the USA and makes detailed presentations on career opportunities within the USDA to minority candidates with undergraduate or graduate degrees, as well as to those seeking postdoctoral positions. To broaden and strengthen the USDA's **outreach efforts to recruit more disadvantaged and underrepresented into the workforce**, Dr. Banuelos is hoping to continue the following efforts with the proposed study:

- (a) establish National Science Scholars Program for underrepresented minorities between USDA and CSU Fresno. This program provides annually three low-income and underrepresented students employment with a scientist mentor at the USDA.
- (b) supervise USDA-ARS summer apprenticeship program for disadvantaged high school students throughout Fresno county.
- (c) execute the American Chemical Society's Project Seed Program, established for providing summer employment to economically disadvantaged high school students throughout Fresno county.
- (d) form group with the USDA called "Hispanic Scientists for a Sustainable Environment"; the mission of the group is to hire members of the Latino community on multi-year field studies.
- (e) mentor at least 5 Science Fair projects for disadvantaged students on various aspects of field studies throughout Fresno county.

2. Dr. Sharmasarkar has several years of experience in environmental research, on-site field works with multiple cropping systems, and extension activities with county agents and growers.

3. Among the eight persons on research team of principal PI, five are members of underrepresented groups. These representatives promote interest among their respective community's which encourages participation for our research projects. For the proposed field study, we conservatively estimate that between 5-7 new hires, apprentices, or interns from underrepresented groups will work and be trained on an annual basis.

4. Information acquired from field sediment study will be readily available to David Cone, **Broadview Water District**; Chase Hurley, **Panoche Water District**; and John Diener, **Grower, Red Rock Ranch, Five Points, CA**. More importantly, field operations, plant selection, and soil and water management strategies will be **demonstrated** to each of them on an annual basis. A practical article will be written and submitted to *California Agriculture* after the first year of operation. More in-depth articles will be submitted to *Agricultural Water Management* and to *Journal of Environmental Quality*.

The results of our research will be **disseminated** in the following ways:

- (a) Compilation of our research may be written up as **two separate Master of Science theses** for the Plant Science Dept. at CSU Fresno in conjunction with Dr. D. Zoldoske.
- (b) Publication in a peer review journal, i.e., *Journal of Environmental Quality*.

- (c) Overview of results will be submitted to: Central Valley Regional Water Quality Control Board; Broadview, Panoche, Mercy Springs, and Firebaugh Canal Water Districts.
- (d) Demonstration on research field site after one year of successful plant growth for growers and Water Districts on the westside of central California.
4. Copies of the letters to Broadview and Panoche Water Districts are enclosed.

#### D. Qualifications of Applicants, Cooperators, and Establishment of Partnerships

- See enclosed resumes and letters.
- This collaborative greenhouse and field research studies between the Water Management Research Lab, west side Water Districts (Broadview and Panoche), growers of Se-laden soils in the west side, and CSU Fresno (Dr. Zoldoske) will utilize the strengths of each institution to jointly address the environmental issue of Se remediation in central California.

#### E. Costs and Benefits

Items (\$)	Year 1		Year 2		Year 3		Total	Total
	Request	Match	Request	Match	Request	Match	Request	Match
A. Salaries and Wages	46000	47000	505000	50000	69750	inhouse	166250	97000
B. Supplies	10000	3000	7000	inhouse	8000	inhouse	25000	3000
C. Equipment	5000	inhouse					5000	
D.	2500		3000		3500		9000	
Services/Consultants								
E. Travel	3000	inhouse	4000	inhouse	5000	inhouse	12000	
F. Other direct Costs	2500	inhouse	2500	inhouse			5000	
G. Net Estimated Costs	69000	50000	67000	50000	86250		222250	
+ 15% indirect costs	10350		10050		12938		33338	
<b>TOTAL COSTS</b>	<b>79350</b>	<b>50000</b>	<b>77050</b>	<b>50000</b>	<b>99188</b>	<b>inhouse</b>	<b>255538</b>	<b>100000</b>

#### Detailed Budget Justification

##### YEAR 1 (July 2001 - June 2002):

##### Salary and Wages (includes fringe benefits)

The senior research associate is requested for three years of the study. He (Dr. Sharmasarkar) will coordinate all aspects of the study and will be assisted by a Ph.D student from UC Davis, who will use parts of the study for her Dissertation. She will be assisted by an undergraduate student. Two other USDA technicians will allocate 50% of their time for field activities related to the field project. An undergraduate student will provide part-time secretarial help specifically for the project. Dr. Banuelos will have three federal technicians available for all activities related to maintenance of the plots and for all soil and plant sampling.

\$25000 for Senior Associate, \$20000 for 2 graduate students, and \$1000 for clerical support.